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(54) Title: IMMUNOTHERAPY FOR AUTOIMMUNE DISEASE

(57) Abstract

A treatment for autoimmune disease based on delivery of antigen by gene gun into the skin, or delivery of antigen-encoding DNA into the skin is described. Delivery of collagen or collagen-coding DNA is useful for treating rheumatoid arthritis, and delivery of myelin basic protein (MBP) or MBP-coding DNA is useful for treating multiple sclerosis. These antigens mitigate cytotoxic responses and elicit antigen desensitization. Other autoimmune diseases can be treated using other antigens.

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IMMUNOTHERAPY FOR AUTOIMMUNE DISEASE

Technical Field

The present invention relates to the use of immunotherapies for the treatment of autoimmune disorders in general and relates, in particular, to the use of techniques of *in situ* antigen or gene delivery for the treatment of autoimmune diseases.

Background of the Invention

Autoimmune Diseases

Autoimmune diseases are characterized by cytotoxic immune responses to epitopes on self antigens natively found in the diseased individual. The immune system of the individual then activates an inflammatory cascade aimed at cells and tissues presenting those specific self antigens. The destruction of the antigen, tissue, cell type, or organ attacked by the individual's own immune system gives rise to the symptoms of the disease.

Clinically significant autoimmune diseases include, for example, rheumatoid arthritis, multiple sclerosis, juvenile-onset diabetes, systemic lupus erythematosus, autoimmune uveoretinitis, autoimmune vasculitis, bullous pemphigus, myasthenia gravis, autoimmune thyroiditis or Hashimoto's disease, Sjogren's syndrome, granulomatous orchitis, autoimmune oophoritis, Crohn's disease, sarcoidosis, rheumatic carditis, ankylosing spondylitis, Grave's disease, and autoimmune thrombocytopenic purpura. *See e.g.*, Paul, W.E. (1993) *Fundamental Immunology*, Third Edition,

Raven Press, New York, Chapter 30, pp. 1033-1097; and Cohen et al. (1994) *Autoimmune Disease Models, A Guidebook*, Academic Press, 1994.

Triggering events for most autoimmune diseases are generally unknown. One theory is that autoimmune disease may be triggered by infection, either clinical or sub-clinical, by an infectious agent which presents the immune system with an epitope that is similar to an epitope natively present on some cell type in the individual. This theory may explain the semi-inheritable nature of many autoimmune diseases since some familial alleles of particular cell surface markers may be more or less similar to the epitope on the actuating infectious agent, and therefore more or less likely to be recognized by antibodies or T-cells that are specific to the infectious agent.

Conventional therapies for autoimmune disease tend to focus on symptomatic relief or generalized immune suppression rather than treatment of the specific underlying immune dysfunction. Such therapeutic approaches are based on the premise that if tolerance can be induced to the specific antigen against which the immune system is acting, progression of the disease should be halted.

Evolving studies of the immune system have led to a generalization of some immune responses into two categories, the categories characterized by the class of helper T-cells activated. These general categories are referred to a Th1 and Th2. Th1 refers to a cytotoxic T lymphocytic sponse (a CTL response), also called a del d hypersensitivity type reaction (DTH) This type o: esponse generally results in destruction of the antigen, cell, tissue, or organ which expresses the antigen. In contrast to the cytotoxic Th1 type of response, the Th2 T

lymphocytic response generally does not participate in delayed type hypersensitivity or cytotoxic responses. Thus, elimination of cytotoxic responses and development of desensitizing responses are considered to be indicia of development of tolerance to the antigen.

An immune response in either of these two categories tends to down-regulate the immune response in the other category. In this regard, interferon-gamma released by cytotoxic T cells inhibits proliferation of Th2 cells, and interleukin-10 released by Th2 cells inhibits the stimulation of cytotoxic cells by monocytic antigen presenting cells. This mutual inhibition tends to favor the development of only one type of response, cytotoxic or desensitizing, to a given antigen. Because of the mutual inhibition and because an animal's immune system is constantly exposed to self antigens, it has been difficult to intervene in the autoimmune process.

Rheumatoid Arthritis

Rheumatoid arthritis is an inflammatory, autoimmune disease affecting multiple systems, but primarily affecting multiple joints. The disease is accompanied by inflammation of the synovial membranes and other joint structures, muscle atrophy, bone erosion and rarefaction, and formation of pannus (fibrovascular inflammatory membrane) on the joint surfaces. Rheumatoid arthritis generally is quite painful and often severely debilitating.

Rheumatoid arthritis is not associated with known infection. The disease is commonly associated with formation of autoantibodies reactive with the subject's own IgG (rheumatoid factor), leading to immunoglobulin aggregates, the role of which in the

disease is unclear. Like many autoimmune diseases, rheumatoid arthritis is more common (~3-fold) in human females than in males. Family studies, including studies of identical and fraternal twins, clearly show that the causation of rheumatoid arthritis is partly genetic and partly non-genetic. The non-genetic component may be either environmental and/or caused by random chance (e.g. somatic mutations, chance biochemical events, stochastic rearrangement of T-cell receptor genes).

Class II genes of the MHC are important in the disease. Approximately 59-70% of patients with clearly diagnosed rheumatoid arthritis have HLA-DR4, compared with ~20-28% of the general population. The most susceptible individuals are heterozygotes with HLA-DR4 (and DQ8) on one copy of chromosome 6 and HLA-DR1 on the other. Recent transgenic mouse studies suggest that HLA-DR and HLA-DQ affect rheumatoid arthritis susceptibility via different mechanisms.

One animal model for the study of human rheumatoid arthritis is an autoimmune arthritis induced in rodents. In order to generate disease, Lewis rats or DBA/1 mice, are intradermally injected with type II collagen (CII) in adjuvant (see, e.g., Stuart et al., *Ann. Rev. Immunol.* 2:199-218 (1984)). This model is referred to as collagen-induced arthritis or CIA.

Prior Studies of Antigen Tolerance in CIA

There are reports in which CII has been used to develop tolerance to the antigen to prevent or ameliorate experimental autoimmune arthritis or human rheumatoid arthritis. Whole CII protein or CII-derived peptides have been given, either orally or by injection. For example, Nagler-Anderson et al., *J. Exp. Med.* 170:1999-2010 (1989) reported that

prophylactic feeding of native, but not denatured, bovine CII reduced the incidence of CIA in DBA/1 mice. In contrast, other experimenters have found that the protein can be native or denatured, and peptides are also effective (Khare et al., *J. Immunol.* 155:3653-3659 (1995)).

The importance of 3-dimensional structure in some protocols suggests that antibodies may play a role in the protection in those instances. Alternatively, the denatured protein may somehow be less available for processing for presentation to T cells. The only immunological variable found to be greatly altered in the Nagler-Anderson study was the reduction in CII-specific IgG2b in the successfully protected mice, compared to those that were not pre-treated or were CII-fed but not successfully protected.

Trentham et al., *Science* 261:1727-1730 (1993) conducted a double-blind placebo-controlled clinical trial of 28 chicken CII-fed and 31 placebo-fed patients with rheumatoid arthritis. The authors reported that clinical and lab variables were similar in the collagen and placebo groups at entry. However, despite the stated random allocation to collagen or placebo groups, the placebo group started out significantly worse than the collagen group by many of the assessment criteria used.

In an unpublished, larger multi-center clinical trial organized by the same group, oral collagen was effective, but only at the lowest dose.

In feeding-induced tolerance experiments using hen egg lysozyme in mice and guinea pig MBP in rats, Friedman and Weiner (*Proc. Natl. Acad. Sci. USA* 91:6688-6692 (1994)) reported a dose-dependent induction of either anergy (at high antigen doses) or active suppression (at low antigen doses). Cells from

low-dose-treated mice (but not those from mice treated with high antigen doses) were able to down-regulate the *in vitro* proliferative response to other antigens (a phenomenon called "bystander suppression"), not given to the cells, as long as the treatment antigen was present in the same culture. Bystander suppression was induced only at low antigen doses. In contrast, D. Wraith (conference presentation cited in *Immunol. Today* 17:9-12 (1996)) was unable to find differences in the effects of high- versus low-dose tolergen feeding on Th1 and Th2 responses.

In summary, desensitization by oral feeding type II collagen is safe and may be clinically effective, although its effectiveness in rheumatoid arthritis patients has not been clearly demonstrated. Its effectiveness is highly dose-dependent, which would clearly complicate its application in the heterogeneous human population.

Another approach in prior investigations of inducing tolerance in the CIA model involves the injection of collagen or collagen-related peptides into an individual. The CII protein or CII-derived peptides have also been used to treat or prevent CIA and in other autoimmune arthritis models (not collagen-induced).

Like the collagen feeding study, a study by Schoen et al., *J. Immunol.* 128:717-719 (1982), using intravenous injection of CII-coupled spleen cells, required native CII to prevent CIA. Another similarity is that both studies decreased the incidence but not the severity of arthritis. Intravenous CII reduced the anti-CII antibody titers approximately 50%, but did not affect the delayed-type hypersensitivity response to CII. The authors suggested that protection was due to a decrease in a class or subclass of autoantibodies. CI could not be

substituted for CII in the desensitization regimen. Another study (Phadke et al., *Arthritis Rheum.* 27:797-806 (1984)) suppressed CIA via intravenous injection of either native or denatured CII, but native CII was much more effective.

In addition to desensitization regimens, a synthetic modified collagen peptide (designed to competitively inhibit binding of the corresponding native collagen peptide to MHC) reduced the incidence and severity of CIA in mice. For clinical application, this approach is less promising than induction of an anti-inflammatory "Th2-like" response since it likely would require the continued presence of large quantities of the synthetic competitor peptide and is not expected to have a bystander effect.

Similar desensitization procedures have been performed using the 65-kDa heat-shock protein from *Mycobacterium tuberculosis*, or Mt Hsp65. Mt Hsp65 is known to cross-react with a component of articular cartilage, now known to be human Hsp60. This and other heat-shock proteins are up-regulated in the joints of arthritic patients. Rheumatoid arthritis patients have T-cell reactivity to Hsp65, and arthritogenic T cell clones from CFA-treated rats recognize a specific epitope of Hsp65. However, Hsp65 emulsified in oil (in place of whole, killed *Mycobacterium tuberculosis* in oil) did not induce adjuvant arthritis (AA), but instead protected against a subsequent attempt to induce adjuvant arthritis with whole *Mycobacterium tuberculosis*. The 9-amino acid immunodominant epitope was similarly protective.

Most of the reports of arthritis prevention via mycobacterial Hsp65 vaccination have used the adjuvant arthritis model in which Hsp65 is implicated as the major antigen. It is important to determine if

the protection is strictly antigen-specific (as with anergy or clonal deletion) or if bystander suppression occurs (as expected with anti-inflammatory cytokines, for example).

Multiple Sclerosis

Multiple sclerosis is a chronic central nervous system disease of considerable medical importance. See Martin et al., Immunological aspects of demyelinating diseases. *Ann. Rev. Immunol.* 10:153-187 (1992). It is the most common demyelinating disorder of the brain and spinal cord. It affects males and females equally, usually beginning between the ages of 20 and 40 years. It can have a subtle onset, with spontaneous remissions and relapses, but its course is often progressive and sometimes relentless.

Pathologically, multiple sclerosis is characterized by localized demyelination of white matter with formation of characteristic plaques. Microscopically, infiltrates of both cytotoxic and helper T cells, as well as macrophages are observed. T cells reactive to myelin basic protein (MBP) and proteolipid protein (PLP) have been found in the cerebrospinal fluid and blood of patients with multiple sclerosis.

The MBP molecule contains multiple epitopes, and those recognized by multiple sclerosis and normal T cells may differ. T cell lines derived from patients with multiple sclerosis recognize an HLA-DR2-restricted epitope in residues 84-106 (Ota et al., *Nature* 346:183-187 (1989)). Additionally, Martin et al., *J. Exp. Med.* 173:19 (1991), identified an epitope in residues 87-106 that was recognized by multiple sclerosis T cells.

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the central nervous system, which, because of its similarities to multiple sclerosis (MS) has often been used as an animal model of that disease (Paterson, P.Y., *Textbook of Immunopathology* (eds Miescher, P.A. and Mueller-Eberhard, H.J.) 179-213 (Grune & Stratton, New York), 1976; Alvord, E.C., Jr. In *Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis* (ed. Alvord, E.C.) 1-511 (Liss, New York, 1984); Steinman, L., *Sci. Am.* 269:106-114 (1993)). The pathology of EAE is characterized by lymphocytic and mononuclear cell inflammation, an increase in blood-brain barrier permeability, and demyelination (Raine et al., *Lab Invest.* 43:150-157 (1980); Paterson et al., *Immunol. Rev.* 55:89-120 (1981)) resulting in partial or complete paralysis and in severe cases death of the affected patient. It is known that neural antigen-specific CD4+ T lymphocytes appear to be the initiators of the response since *in vivo* depletion of CD4+ T cells inhibits induction of EAE (Waldor et al., *Science* 227:415-417 (1985)) and CD4+ T cell lines and clones can passively transfer disease (Holda et al., *Eur. J. Immunol.* 12:453-455 (1982); Ben-Nun et al., *J. Immunol.* 129:303-308 (1982)).

Autoimmune encephalomyelitis, which occurs in people either as a result of vaccination or following viral infections of the central nervous system (also called post-infectious encephalomeylitis or acute disseminated encephalomyelitis), appears to be analogous to the actively induced form of EAE found in experimental animals in that it is characterized by infiltration of inflammatory cells into the central nervous system, breakdown of the blood brain barrier and demyelination resulting in, *inter alia*, partial or complete paralysis and in severe cases death

(Anderson, J.R., *Current Topics in Pathology* 76:23-60 (1988); Rabinowitz et al., *J. Neurol. Sci.* 60:393-400 (1983)). Like the animal model, antibodies against the neural myelin basic protein are found in patients who survive this disease (Anderson, J.R., (1988) *supra*; Rabinowitz et al., (1983), *supra*).

The autoreactive T cells that induce organ specific autoimmune diseases, including EAE, generally display a helper T cell type 1 (Th1) phenotype (Miller et al., *Immunol. Today* 15:356 (1994)). The adoptive transfer of myelin-reactive Th1 cells but not Th2 cells can induce EAE and cytokines associated with a Th1 type response are present in the inflammatory lesions of the central nervous system of animals affected by EAE, whereas cytokines associated with a Th2 type are absent from such lesions. Based on these findings, it has been suggested that Th1 cytokines may play a role in the pathology of EAE (Kuchroo et al., *J. Immunol.* 151:3776 (1992); Zamvil et al., *Ann. Rev. Immunol.* 8:579 (1990)).

In contrast, regulatory cells involved in suppressing the development of EAE are those associated with a cytokine profile of the Th2 phenotype, and recovery from EAE is associated with the presence of Th2 cells and cytokines in the CNS (Khoury et al., *J. Exp. Med.* 148:3776 (1992); Kennedy et al., *J. Immunol.* 149:2496 (1992)). The latter findings suggest that the preferential induction and activation of Th2 type cells by the appropriate antigen could potentially be useful in the prevention of EAE and other autoimmune diseases caused by Th1 cells.

Diabetes Mellitus

Diabetes mellitus is a disorder of glucose metabolism due to a relative or absolute lack of

insulin (reviewed in Atkinson et al., *Sci. Am.* 263:62-71 (1990) and further described in Paul, W.E., *Fundamental Immunology*, 3rd edition, Raven Press, New York, 1993). There are two principal forms of the disease, type I and type II diabetes. Type I diabetes is characterized by the patient's dependency upon exogenous insulin to maintain normal glucose metabolism. Type I diabetes is an autoimmune disease that destroys the insulin-producing beta cells of the islets of Langerhans in the pancreas. The specific lesion, called "insulitis" is characterized by an infiltration of the islets of Langerhans by mononuclear cells, mainly CD8+ T cells, and to a lesser extent by CD4+ T cells (Rossini et al., *Annu. Rev. Immunol.* 3:289-320 (1985)).

Autoantibodies against islet cells are characteristic in type I diabetes, some bind to cytoplasmic constituents, and others bind to proteins on the beta-cell membrane. A notable feature of these anti-islet cell antibodies is that they precede the onset of the disease, and some subsets (such as complement-fixing IgG anti-islet cell antibodies) can predict the disease in susceptible individuals. Antibodies against a cytoplasmic 64 kD beta-cell protein occur in about 80% of newly diagnosed patients, and over 90% of patients have antibodies against particular tryptic fragments of the protein (Christie et al., *J. Exp. Med.* 172:789-794 (1990)). These antibodies may be present for years before the onset of disease, and thus may be predictive markers. Another potential immunogen is a 38-kD beta-cell granule protein. It stimulates *in vitro* proliferation of T cells from diabetics (Roep et al., *Nature* 345:632-634 (1990)). This immune response may provide the link between cytotoxic T-cell responses and type I diabetes

Unfortunately, current therapy for type I diabetes primarily consists of injection of insulin. However, the ability to predict the disease by means of assays for anti-islet cell antibodies, along with the availability of tests for residual beta-cell function, raises the possibility of preventive therapy. This possibility is of substantial interest because in many cases, almost all beta-cells have been destroyed by the time of diagnosis. Clinical trials point to the efficacy of immunosuppressive drugs in type I diabetes (Schwartz, R.P., *New. Engl. J. Med.* 319:649 (1988)), but whether these potent agents should be used in suspected pre-diabetics is debatable. Thus, a more acceptable preventive therapy would be desirable.

Summary of the Invention

The present invention provides an effective method for treating an autoimmune disease. The method is based on the induction of self antigen desensitization in an individual by introduction of the self antigen, or a gene coding therefor, into a cell of the individual. The antigen is selected on the basis of its involvement in the autoimmune process.

Accordingly, in one embodiment, the subject invention is directed to a method for treating or preventing an autoimmune disease in a mammal comprising the steps of:

(a) providing a particle coated with an antigen against which an immune response is mounted in the autoimmune disease, wherein the particle is suitable for delivery into a recipient cell, the particl having an averag diameter of about 0.5 to about 5 μm and a density sufficient to allow delivery into the recipient cell;

(b) delivering the particle into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

In another embodiment, the subject invention is directed to a method for treating or preventing an autoimmune disease in a mammal comprising the steps of:

(a) providing a nucleic acid construct which comprises a coding sequence for an antigen against which an immune response is mounted in the autoimmune disease, operably linked to control elements such that the coding sequence can be transcribed and translated in a recipient cell;

(b) delivering the nucleic acid construct into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

In a related embodiment, the invention is directed to a method for treating or preventing an autoimmune disease in a mammal, wherein the method comprises:

(a) coating a particle with a nucleic acid construct which comprises a coding sequence for an antigen against which an immune response is mounted in the autoimmune disease, operably linked to control elements such that the coding sequence can be transcribed and translated in a recipient cell, wherein the particle has an average diameter of about 0.5 to about 5 μm and a density sufficient to allow delivery into the recipient cell;

(b) delivering the particle into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

In particularly preferred embodiments, the cytotoxic immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-2, interferon-gamma and tumor necrosis factor. The desensitizing immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-4, interleukin-5, interleukin-6, and interleukin-10. In one related embodiment, the desensitizing immune response is characterized by augmented production of one or more mediators selected from the group consisting of interleukin-4, interleukin-10, and, surprisingly, interferon-gamma.

In additional embodiments, the autoimmune disease is rheumatoid arthritis and the antigen is selected from the group consisting of collagen, the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, and epitopes thereof.

In yet further embodiments, the autoimmune disease is multiple sclerosis and the antigen is selected from the group consisting of myelin basic protein, myelin oligodendrocyte glycoprotein, proteolipid protein, and epitopes thereof.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Drawings

Fig. 1 depicts the results of an experiment where type II collagen (CII) was delivered to mice.

Fig. 2 shows IgG subclass responses in CII-challenged mice.

Fig. 3 shows IgG subclass responses in Hsp65-DNA-pretreated, CII-challenged mice.

Fig. 4 shows the protocol for immunizing rats for treatment of experimental autoimmune encephalomyelitis (EAE).

Detailed Description of the Preferred Embodiments

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular antigens, antigen-coding nucleotide sequences, or the specifically enumerated diseases. It is also understood that different applications of the disclosed methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more such agents, reference to "a particle" includes reference to mixtures of two or more particles, and the like.

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the

art to which the invention pertains. The following terms are intended to be defined as indicated below.

As used herein, the term "autoimmune disease" means a set of sustained organ-specific or systemic clinical symptoms and signs associated with altered immune homeostasis that is manifested by qualitative and/or quantitative defects of expressed autoimmune repertoires (*Autoimmunity Physiology and Disease*, Coutinho and Kazatchkine, eds, Wiley-Liss, 1993, Chapter 27, page 433).

An "antigen" refers to any agent, generally a macromolecule, which can elicit an immunological response in an individual. The immunological response may be of B- and/or T-lymphocytic cells. The term may be used to refer to an individual macromolecule or to a homogeneous or heterogeneous population of antigenic macromolecules. As used herein, "antigen" is used to refer to a protein molecule or portion thereof which contains one or more epitopes.

The term "epitope" generally refers to the site on an antigen to which a specific antibody molecule binds. The identification of epitopes which are able to elicit an antibody response is readily accomplished using techniques well known in the art. See, e.g., Geysen et al. *Proc. Natl. Acad. Sci. USA* (1984) 81:3998-4002 (general method of rapidly synthesizing peptides to determine the location of immunogenic epitopes in a given antigen); U.S. Patent No. 4,708,871 (procedures for identifying and chemically synthesizing epitopes of antigens); and Geysen et al., *Molecular Immunology* (1986) 23:709-715 (technique for identifying peptides with high affinity for a given antibody). T-cell epitopes are generally those features of a peptide structure capable of inducing a T-c ll response. In this regard, it is accepted in the art that T-cell epitopes comprise

linear peptide determinants that assume extended conformations within the peptide-binding cleft of MHC molecules, (Unanue et al., *Science* (1987) 236:551-557). As used here, an epitope is generally a peptide having about 3-5, preferably 5-10 or more amino acid residues. Particular epitopes useful in the practice of the invention include, but are not limited to, the 9 amino acid immunodominant epitope of Hsp65 and the epitope present in residues 84-106 of the MBP molecule.

The term "self antigen," which is used interchangeably herein with the term "autoantigen," means an antigen, or a molecule capable of being recognized during an immune response, that is normally part of the individual. This is in contrast with antigens which are foreign, or exogenous, which are not normally part of the individual's milieu. Each autoimmune disease is characterized by an immune response directed at a self antigen. Normally, there are no active immune responses to self antigens, and no symptoms appear. With the development of an immune response to a self antigen, autoimmune diseases may appear. Autoimmune diseases present clinically with different symptoms depending upon the specific self antigen against which an immune response is raised. This immune response results in the destruction of the structure containing the self antigen, and it is the loss of that structure with concurrent loss of that structure's normal function which results in symptoms of autoimmune disease.

As used herein, the term "anergy" means a reversible antiproliferative state which results in decreased responsiveness of an immune cell or cells to an antigen.

The term "antigen desensitization" refers to the process of decreasing an immune response by

delivering to the cell or animal, over a period of time, the antigen against which an immune response is mounted. With repeated exposure of the immune cells to the antigen, a decrease in the cytotoxic response is seen. Such desensitization includes, but is not limited to, a switch from a "Th1" to a "Th2" response. Antigen desensitization may result in the cessation of the autoimmune process, and may ultimately result in the replenishment of the individual's self antigen, and the associated structures.

The term "Th1" means that response of T-lymphocytes to antigen characterized by, but not limited to, T-lymphocyte secretion of one or more of the following mediators: interleukin-2 (IL-2); interferon-gamma (IFN-gamma); and tumor necrosis factor-beta (TNF-beta). The lymphokines and cells activated during a Th1 response are primarily mediators of delayed hypersensitivity type (DTH) or cytotoxic reactions. It is understood that other mediators may be released without changing the meaning of "Th1."

The term "Th2" means that response of T-lymphocytes to antigen characterized by, but not limited to, T-lymphocyte secretion of one or more of the following mediators: interleukin-4 (IL-4); interleukin-5 (IL-5); interleukin-6 (IL-6); and interleukin-10 (IL-10). The lymphokines produced by Th2 cells are primarily mediators of helper T-cell function for B-cell antibody production, and are not generally part of the delayed hypersensitivity type response (DTH) or of the cytotoxic response. Th2 responses are generally desensitizing to the antigen. It is understood that other mediators may be released without changing the meaning of "Th2."

By "vector" is meant any genetic element, such as a plasmid, phage, transposon, cosmid,

chromosome, virus, virion, etc., which is generally capable of replication when associated with the proper control elements and present in an appropriate host cell.

"Gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), integration of transferred genetic material into the genomic DNA of host cells, or eventual loss of the DNA through degradative processes.

A "nucleotide sequence" or a "nucleic acid molecule" refers to DNA and RNA sequences. The term captures molecules that include any of the known base analogues of DNA and RNA.

A "coding sequence" or a sequence which "encodes" a particular polypeptide, is a nucleic acid sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are conventionally determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eukaryotic mRNA, genomic DNA sequences from procaryotic or eukaryotic DNA, and even synthetic DNA sequences. In eukaryotic DNA, a transcription termination sequence will usually be located 3' to the coding sequence.

The term DNA "control sequences" refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of r plication, internal

ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected gene is capable of being transcribed and translated in an appropriate recipient cell.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

B. General Methods

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

As explained above, the present invention allows for the treatment and/or prevention of a wide

variety of autoimmune diseases including, but not limited to, rheumatoid arthritis, multiple sclerosis, juvenile-onset diabetes, systemic lupus erythematosus, autoimmune uveoretinitis, autoimmune vasculitis, bullous pemphigus, myasthenia gravis, autoimmune thyroiditis or Hashimoto's disease, Sjogren's syndrome, granulomatous orchitis, autoimmune oophoritis, Crohn's disease, sarcoidosis, rheumatic carditis, ankylosing spondylitis, Grave's disease, and autoimmune thrombocytopenic purpura. The method utilizes autoantigen desensitization carried out using, for example, particle-mediated delivery techniques (e.g., gene gun delivery). A wide variety of nucleic acid molecules or peptide antigens can be delivered using the methods of the invention. The particular molecule selected depends on the autoimmune disease being treated, for example, if the autoimmune disease to be treated is rheumatoid arthritis, the autoantigen can be selected from the group of collagen, the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, epitopes thereof capable of eliciting the appropriate immune response, and nucleic acid molecules encoding the same. If the autoimmune disease is multiple sclerosis, the autoantigen can be selected from the group of myelin basic protein, myelin oligodendrocyte protein, proteolipid protein, epitopes thereof, and nucleic acid molecules encoding the same, as well as coding sequences encoding the entire cDNA library of human oligodendrocytes or an oligodendrocyte gene library.

Generally, nucleic acid molecules used in the subject methods contain coding regions with suitable control sequences and, optionally, ancillary therapeutic nucleotide sequences. The nucleic acid molecules are pr pared in the form of vectors which include the necessary elements to direct transcription

and translation in a target cell. If expression is desired using the host's enzymes (such as by the use of endogenous RNA polymerase), the gene or genes will be present in the vectors operatively linked to control sequences recognized by the particular host, or even particular cells within the host. Thus, eukaryotic control elements will be present for expression in mammalian hosts. Such sequences are known in the art and are discussed more fully below.

In order to augment anti-inflammatory responses, the above-described autoantigens can be administered in conjunction with ancillary substances, such as pharmacological agents, adjuvants, cytokines, or in conjunction with delivery of vectors encoding cytokines. Furthermore, to avoid the possibility of eliciting unwanted anti-self cytokine responses when using cytokine codelivery, chemical immunomodulatory agents such as the active form of vitamin D3 can also be used. In this regard, 1,25-dihydroxy vitamin D3 has been shown to exert an adjuvant effect via intramuscular DNA immunization.

Ancillary nucleic acid sequences coding for peptides known to stimulate, modify, or modulate a host's immune response, can be coadministered with the above-described antigens. Thus, genes encoding one or more of the various cytokines (or functional fragments thereof), such as the interleukins, interferons, and colony stimulating factors, will find use with the instant invention. The gene sequences for a number of these substances are known. For example, genes encoding IL-4 and IL-10 can be codelivered with autoantigen preparations. Further, the inventors have discovered that autoantigen delivery to the epidermis elicits an autoantigen-specific anti-inflammatory immune response that is characterized by suppression of IgG2 antibody response, T cell proliferation and

IL-2 production, coupled with augmentation of IL-4, IL-10 and interferon gamma (IFN-γ) production. The suppression of IgG2 and IL-2 production, coupled with augmented production of IL-4 and IL-10 is reminiscent of a Th1 to Th2 shift. However, the corresponding augmentation of IFN-γ production is a surprising and unexpected event in an anti-inflammatory response. Elevated IFN-γ responses may thus play a role in the protective mechanism, rather than contributing to autoimmune disease as previously believed. In this regard, recent reports have demonstrated that IFN-γ plays an important down-regulatory role in both the induction and effector phases of myelin oligodendrocyte glycoprotein-induced EAE in mice. Willenborg et al., (1996) *J. Immunol.* 157:3223-3227. Thus, in one embodiment of the invention, delivery of an autoantigen is coupled with codelivery of one or more of the following immunological response modifiers: IL-4; IL-10; and IFN-γ.

In addition, ancillary agents can be used to enhance desensitization, such as where the cytotoxic response is pharmacologically suppressed. In this regard, CIA and AA in rodents, and RA in humans, each appear to be mediated by cytotoxic immune responses. This observation is supported by data showing that both anti-IL-2 receptor monoclonal antibodies and IL-4 can suppress CIA in mice, while IL-12 and interferon-gamma exacerbate disease. Thus, in another embodiment of the invention, autoantigen-specific TH1 immunity can be transiently suppressed using suitable pharmacological agents such as IL-4, anti-IL-2 receptor antibody molecules, anti-TNF-alpha antibodies, and anti-CD4 antibody molecules.

Other approaches to systemically modulating inflammatory cytotoxic immune responses during desensitization to autoantigens is to pretreat and/or

codeliver a vector encoding a known desensitizing antigen such as HIV-1 gp120. In this manner, systemic induction of desensitization may transiently ameliorate active disease symptoms since HIV-1 infection has been reported to have similar effects in human rheumatoid arthritis patients. Alternatively, other means of systemically suppressing inflammatory responses prior to or during active autoantigen delivery is to administer anti-inflammatory compounds such as prednisone or methotrexate. These compounds are currently used to suppress rheumatoid arthritis and other inflammatory diseases in humans, and could sufficiently alter the systemic cytokine balance to enable elicitation of the desired anti-inflammatory effects via autoantigen treatment. Furthermore, in order to take advantage of a bystander effect in the treatment of RA, vectors encoding other articular cartilage antigens, such as the cartilage proteoglycan core proteins, aggrecan and lumican, can be codelivered with RA-specific autoantigens to help decrease cytotoxic responses. In this way, induction of an immune response by T lymphocytes recognizing new self antigens in local sites of inflammation results in the local production of anti-inflammatory cytokines, and ameliorates pathology and symptoms of disease. Expression vectors encoding such articular cartilage antigens can be constructed using, for example, cDNA sequences of human and other vertebrate proteoglycan core protein genes, which are readily available.

Modes of carrying out the invention are described more fully below.

Isolation of Genes and Construction of Vectors

Nucleotide sequences selected for use in the present invention can be derived from known sources,

for example, by isolating the same from cells containing a desired gene or nucleotide sequence using standard techniques. Similarly, the nucleotide sequences can be generated synthetically using standard modes of polynucleotide synthesis that are well known in the art. See, e.g., Edge et al., *Nature* 292:756 (1981); Nambair et al., *Science* 223:1299 (1984); Jay et al., *J. Biol. Chem.* 259:6311 (1984). Generally, synthetic oligonucleotides can be prepared by either the phosphotriester method as described by Edge et al. (*supra*) and Duckworth et al., *Nucleic Acids Res.* 9:1691 (1981), or the phosphoramidite method as described by Beaucage et al., *Tet. Letts.* 22:1859 (1981), and Matteucci et al., *J. Am. Chem. Soc.* 103:3185 (1981). Synthetic oligonucleotides can also be prepared using commercially available automated oligonucleotide synthesizers. The nucleotide sequences can thus be designed with appropriate codons for a particular amino acid sequence. In general, one will select preferred codons for expression in the intended host. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. *See, e.g.*, Edge et al. (*supra*); Nambair et al. (*supra*) and Jay et al. (*supra*).

Another method for obtaining nucleic acid sequences for use herein is by recombinant means. Thus, a desired nucleotide sequence can be excised from a plasmid carrying the same using standard restriction enzymes and procedures. Site specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by manufacturers of commercially available restriction enzymes. If

d sired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques.

Restriction cleaved fragments may be blunt ended by treating with the large fragment of *E. coli* DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using standard techniques. The Klenow fragment fills in at 5' single-stranded overhangs but digests protruding 3' single strands, even though the four dNTPs are present. If desired, selective repair can be performed by supplying only one, or several, selected dNTPs within the limitations dictated by the nature of the overhang. After Klenow treatment, the mixture can be extracted with e.g. phenol/chloroform, and ethanol precipitated. Treatment under appropriate conditions with S1 nuclease or BAL-31 results in hydrolysis of any single-stranded portion.

Yet another convenient method for isolating specific nucleic acid molecules is by the polymerase chain reaction (PCR). Mullis et al., *Methods Enzymol.* 155:335-350 (1987). This technique uses DNA polymerase, usually a thermostable DNA polymerase, to replicate a desired region of DNA. The region of DNA to be replicated is identified by oligonucleotides of specified sequence complementary to opposite ends and opposite strands of the desired DNA to prime the replication reaction. The product of the first round of replication is itself a template for subsequent replication, thus repeated successive cycles of replication result in geometric amplification of the DNA fragment delimited by the primer pair used. This method also allows for the facile addition of nucleotide sequences onto the ends of the DNA product by incorporating these added sequences onto the oligonucleotide primers (see, e.g., *PCR Protocols, A*

Guide to Methods and Applications, Innis et al (eds) Harcourt Brace Jovanovich Publishers, NY (1994)).

Once coding sequences for desired proteins have been prepared or isolated, such sequences can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Ligations to other sequences are performed using standard procedures, known in the art.

Selected nucleotide sequences can be placed under the control of regulatory sequences such as a promoter or ribosome binding site (collectively referred to herein as "control" elements), so that the sequence encoding the desired protein is transcribed into RNA in the host tissue transformed by a vector containing this expression construct.

The choice of control elements will depend on the host being treated and the type of preparation used. Thus, if the host's endogenous transcription and translation machinery will be used to express the proteins, control elements compatible with the particular host will be utilized. In this regard, several promoters for use in mammalian systems are known in the art and include, but are not limited to, promoters derived from SV40, CMV, HSV, RSV, MMTV, among others.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of protein sequences encoded by the delivered nucleotide sequences. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expr ssion of a coding sequence to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound.

Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate control and, optionally, regulatory sequences such that the positioning and orientation of the coding sequence with respect to the control sequences allows the coding sequence to be transcribed under the "control" of the control sequences (i.e., RNA polymerase, which binds to the DNA molecule at the control sequences, transcribes the coding sequence). Modification of the sequences encoding the particular protein of interest may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it is attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

Preparation of Peptide Antigens

Peptide antigens can be produced using a variety of methods known to those skilled in the art. In particular, the antigens can be isolated directly from native sources, using standard purification techniques. Alternatively, the antigens can be recombinantly produced using expression systems as described above and purified using known techniques. The peptide antigens can also be synthesized, based on described amino acid sequences or amino acid sequences derived from the DNA sequence of a nucleic acid molecule of interest, via chemical polymer syntheses

such as solid phase peptide synthesis. Such methods are known to those skilled in the art. See, e.g., J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology, supra*, Vol. 1, for classical solution synthesis.

Administration of Nucleic Acid Preparations

Methods for delivering nucleic acid preparations are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. A number of viral based systems have been developed for transfer into mammalian cells. For example, retroviral systems have been described (U.S. Patent No. 5,219,740; Miller et al., *BioTechniques* 7:980-990 (1989); Miller, A.D., *Human Gene Therapy* 1:5-14 (1990); Scarpa et al., *Virology* 180:849-852 (1991); Burns et al., *Proc. Natl. Acad. Sci. USA* 90:8033-8037 (1993); and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* 3:102-109 (1993). A number of adenovirus vectors have also been described, see e.g., Haj-Ahmad et al., *J. Virol.* 57:267-274 (1986); Bett et al., *J. Virol.* 67:5911-5921 (1993); Mittereder et al., *Human Gene Therapy* 5:717-729 (1994); Seth et al., *J. Virol.* 68:933-940 (1994); Barr et al., *Gene Therapy* 1:51-58 (1994); Berkner, K.L., *BioTechniques* 6:616-629 (1988); and Rich et al., *Human Gene Therapy* 4:461-476 (1993). Adeno-associated virus (AAV) vector systems have also been developed for nucleic acid delivery. AAV vectors

can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* 8:3988-3996 (1988); Vincent et al., *Vaccines 90* (Cold Spring Harbor Laboratory Press) (1990); Carter, B.J., *Current Opinion in Biotechnology* 3:533-539 (1992); Muzyczka, N., *Current Topics in Microbiol. and Immunol.* 158:97-129 (1992); Kotin, R.M., *Human Gene Therapy* 5:793-801 (1994); Shelling et al., *Gene Therapy* 1:165-169 (1994); and Zhou et al., *J. Exp. Med.* 179:1867-1875 (1994).

The nucleic acid molecule of interest can also be delivered without a viral vector. For example, the molecule can be packaged in liposomes prior to delivery to the subject. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug et al., *Biochim. Biophys. Acta.* 1097:1-17 (1991); Straubinger et al., in *Methods of Enzymology*, Vol. 101, pp. 512-527 (1983).

The nucleic acid preparations of the present invention may also be encapsulated, adsorbed to, or associated with, particulate carriers for delivery to suitable recipient cells. Exemplary particulate systems and polymers include, for example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* 5:163-187 (1990), for a review of delivery systems useful for transfer of nucleic acid molecules.

Preparation of Coated Carrier Particles

In preferred embodiments of the invention, the autoantigen preparations are administered using particle mediated delivery techniques. Thus, once prepared and suitably purified, the above-described nucleic acid molecules and peptide antigens can be coated onto carrier particles using a variety of techniques known in the art. Carrier particles are selected from materials which have a suitable density in the range of particle sizes typically used for intracellular delivery from a gene gun device. The optimum carrier particle size will, of course, depend on the diameter of the target cells.

For the purposes of the invention, tungsten, gold, platinum and iridium carrier particles can be used. Tungsten and gold particles are preferred. Tungsten particles are readily available in average sizes of 0.5 to 2.0 μm in diameter. Although such particles have optimal density for use in particle acceleration delivery methods, and allow highly efficient coating with DNA, tungsten may potentially be toxic to certain cell types. Gold particles or microcrystalline gold (e.g., gold powder A1570, available from Engelhard Corp., East Newark, NJ) will also find use with the present methods. Gold particles provide uniformity in size (available from Alpha Chemicals in particle sizes of 1-3 μm, or available from Degussa, South Plainfield, NJ in a particle size of 0.95μm) and reduced toxicity. Microcrystalline gold provides a diverse particle size distribution, typically in the range of 0.5-5 μm. However, the irregular surface area of microcrystalline gold provides for highly efficient coating with DNAs.

A number of methods are known and have been described for coating or precipitating DNA or RNA onto

gold or tungsten particles. Most such methods generally combine a predetermined amount of gold or tungsten with plasmid DNA, $CaCl_2$ and spermidine. The resulting solution is vortexed continually during the coating procedure to ensure uniformity of the reaction mixture. After precipitation of the DNA, the coated particles can be transferred to suitable membranes and allowed to dry prior to use, coated onto surfaces of a sample module or cassette, or loaded into a delivery cassette for use in particular gene gun instruments.

Peptide or protein antigens can also be coated onto suitable carrier particles, e.g., gold or tungsten. For example, peptides can be attached to the carrier particle by simply mixing the two components in an empirically determined ratio, by ammonium sulfate precipitation or other solvent precipitation methods familiar to those skilled in the art, or by chemical coupling of the peptide to the carrier particle. The coupling of L-cysteine residues to gold has been previously described (Brown et al., *Chemical Society Reviews* 9:271-311 (1980)). Other methods include, for example, dissolving the peptide antigen in absolute ethanol, water, or an alcohol/water mixture, adding the solution to a quantity of carrier particles, and then drying the mixture under a stream of air or nitrogen gas while vortexing. Alternatively, the peptide antigens can be dried onto carrier particles by centrifugation under vacuum. Once dried, the coated particles can be resuspended in a suitable solvent (e.g., ethyl acetate or acetone), and triturated (e.g., by sonication) to provide a substantially uniform suspension.

Administration of Coated Particles

Following their formation, carrier particles coated with either nucleic acid preparations, or

peptide or protein antigen preparations, are deliver d to mammalian tissue using particle-mediated delivery techniques.

Various particle acceleration devices suitable for particle-mediated delivery are known in the art, and are all suited for use in the practice of the invention. Current device designs employ an explosive, electric or gaseous discharge to propel the coated carrier particles toward target cells. The coated carrier particles can themselves be releasably attached to a movable carrier sheet, or removably attached to a surface along which a gas stream passes, lifting the particles from the surface and accelerating them toward the target. An example of a gaseous discharge device is described in U.S. Patent No. 5,204,253. An explosive-type device is described in U.S. Patent No. 4,945,050. One example of an electric discharge-type particle acceleration apparatus is the ACCELL® instrument manufactured by Auragen, Inc., Madison, WI, which instrument is described in U.S. Patent No. 5,120,657. Another electric discharge apparatus suitable for use herein is described in U.S. Patent No. 5,149,655.

The coated particles are administered to the subject to be treated in a manner compatible with the dosage formulation, and in an amount that will be prophylactically and/or therapeutically effective. The amount of the composition to be delivered which, in the case of nucleic acid molecules is generally in the range of from 0.001 to 10.0 μg, more preferably 0.01 to 10.0 μg of nucleic acid molecule per dose, and in the case of peptide or protein molecules is 1 μg to 1 mg, more preferably 1 to 50 μg of peptide, depends on the subject to be treated. The exact amount nec ssary will vary depending on the age and general condition of the individual to be treated, the

severity of the autoimmune condition being treated and the particular nucleotide sequence or peptide antigens selected, the site of administration, as well as other factors. An appropriate effective amount can be readily determined by one of skill in the art upon reading the instant specification.

Thus, a "therapeutically effective amount" of the antigens, or nucleic acids coding therefor, will be sufficient to bring about treatment or prevention of autoimmune disease or condition symptoms, and will fall in a relatively broad range that can be determined through routine trials.

The coated particles are delivered to suitable target cells, most preferably to cells of the skin, such as the epidermis. In this regard, it has been found that the introduction of autoantigen into the epidermis of a mammal by gene gun can be performed in such a way so as to induce desensitization and decrease in cytotoxic T-cell responses. It may alternatively be desired to deliver the particles to mucosal tissue cells, in order to bring about mucosal, humoral and cellular immune responses in the treated subject.

Delivery of the particles is timed and repeated in such a manner so as to facilitate the suppression of cytotoxic immune responses and facilitate the creation of a desensitizing-type response which will, in turn, inhibit the arousal of a cytotoxic inflammatory immune response. One way to elicit such a desensitizing response in the practice of the invention is to deliver antigen to a subject over a relatively prolonged period of time, for example over a period of several weeks. Such delivery may result in T-cell anergy, a state of reversible non-proliferation. In this condition, cytotoxic T

cells do not proliferate and the cytotoxic effects of autoimmune diseases are minimal.

On the other hand, multiple and frequent deliveries of self antigen-encoding expression vectors via gene gun delivery to the epidermis generally provide transient bursts of localized antigen production that result in the induction of Th2-like responses characterized by a preponderance of IgG1 antibodies, diminished interferon-gamma production, suppressed CTL activity, and elevated IL-4 production. Thus, desensitization to antigens can be brought about using repeated gene gun delivery of antigens directly into the epidermis.

Thus, in one embodiment, a method for the treatment of rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases is provided which involves an active, rather than passive immunotherapeutic strategy. This approach is based on the ability of epidermal antigen delivery to desensitize the immune system to the self antigen, and thereby eliminate the pro-inflammatory cytokine milieu at its source. As stated above, an example of this strategy involves the use of oral tolerance to anergize or actively suppress pro-inflammatory cytotoxic responses. These studies, and those involving the parenteral administration of self or "self-like" antigens in the rodent adjuvant arthritis and collagen-induced arthritis models support the concept of the "bystander" effect as being potentially useful in the treatment of autoimmune disease, particularly in the treatment of human rheumatoid arthritis.

While it is easy to envision how prior collagen and mycobacterial heat shock protein immunizations (Hsp65) can prot ct against CIA and AA, respectively, evid nce for the bystander effect has

come from studies showing that oral collagen administration can suppress AA induction, and that immunization with Hsp65 can suppress arthritis induced via lipoidal amine administration.

Because of the apparent bystander phenomenon, strategies based on active immunotherapy do not require that one know the identity of the specific self antigen(s) responsible for disease induction. Continued maintenance of a desensitizing immune response by the judicious administration of appropriate antigens that participate in the bystander effect can keep the disease process in check for an indefinite period of time. Support for such an approach can be found in the observation of partial remission of rheumatoid arthritis in certain patients during pregnancy and early HIV infection.

Unlike oral tolerance studies in mice and rats, where only partial protection against subsequent autoimmune disease development has been observed, it has been found by the inventors herein that using particle-mediated techniques to deliver type II collagen (as protein), or a mycobacterial heat shock protein gene, results in 100% protection against disease development in a murine CIA model. These results demonstrate that gene gun delivery provides for the heretofore unattainable suppression of antigen-specific pro-inflammatory immune responses.

Additionally, the present inventors have discovered that treatment of rats which are susceptible to the development of acute encephalomyelitis, via delivery of DNA encoding the gene for myelin basic protein (a major constituent of the myelin sheet surrounding neurons in the central nervous system), protects animals against developing encephalomyelitis. Furthermore, immunization by the nucleic acid-based methods described herein protects

such animals from autoimmune disease of the central nervous system by inducing a switch in the type of immune response which they exhibit after antigen challenge with myelin basic protein (Paterson, P.Y., *Textbook of Immunopathology* (eds Miescher, P.A. and Meuller-Eberhard, H.J.) 179-213 (Grune & Stratton, New York, 1976).

In contrast to the results seen in animals treated with DNA coding for myelin basic protein, control animals receiving plasmid DNAs not containing the gene for myelin basic protein were not protected from developing EAE. While it is not intended that the present invention should be restricted in any way by a theoretical explanation of the mechanism of action of the efficacy, it is believed that such treatment may exert its beneficial effects by desensitizing the animal and decreasing the cytotoxic type of immune response.

C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1

Prevention of Collagen Induced Arthritis with Type II Collagen

In the first attempt to determine if gene gun treatment can elicit protection against the

development of collagen-induced arthritis (CIA), an experiment was designed to determine the following:

(1) whether intradermal injection of bovine type II collagen (CII) protein could elicit CIA in DBA/1 mice;

(2) whether gene gun-mediated delivery of CII to the epidermis would not elicit a similar disease on its own; and

(3) whether gene gun-mediated delivery of CII to the epidermis could elicit CII-specific Th2-like immune responses that might protect against CIA development.

Bovine CII (Sigma, St. Louis) was coated onto 0.95 μm gold particles via ammonium sulfate precipitation. CII-coated gold particles were washed with ethanol and loaded into individual gene gun shots as previously described (Fuller et al., *supra*). Each shot contained approximately 5 μg CII protein and 0.5 mg of gold. Epidermal delivery of protein-coated gold particles was performed using the hand-held, helium driven Accell gene delivery system (PCT pat. appl. WO 95/19799). Twenty 6 week old female DBA/1 mice each received single CII gene gun shots to the abdominal epidermis on days 0, 14 and 35 after fur removal by clipping. Half of these animals, and 9 additional naive mice, received subcutaneous injections of 200 μg CII protein in Freund's complete adjuvant, containing 100 μg *M. tuberculosis*, on day 28. Incidence of arthritis in each group was plotted. Disease was scored as described by Khare et al., *J. Immunol.* 155:3653-3659 (1995), in which mice were monitored for redness or swelling in paws or toes, severe swelling and joint deformity, and joint ankylosis.

Figure 1 shows the incidence of arthritic disease in these three groups of animals as a function of time. While subcutaneous CII injections in

complete Freund's adjuvant induced arthritic disease in 8 of 9 animals that received the CII/Freund's injections only (■), no animals in the groups given CII by gene gun (●), or CII/Freund's by gene gun (△), exhibited any symptoms of arthritis. These data demonstrate that gene gun-mediated administration of CII protein to the epidermis not only failed to elicit CIA on its own, but completely protected against the development of CIA following CII/Freund's challenge.

The dramatic protection against the development of any CIA disease symptoms via the delivery of three gene gun-mediated CII doses (2 before and 1 after challenge) suggests that the gene gun-treated animals reacted immunologically to the epidermal delivery of CII protein. To determine if measurable differences could be detected, bovine CII-specific IgG1, IgG2a, and IgG2b responses were measured in all animals six weeks after challenge (day 70); these data are shown in Figure 2. Figure 2 plots the IgG subclass responses in CII-challenged mice. Serum samples from mice immunized as described above were collected on day 70 (42 days after subcutaneous CII/Freund's challenge). CII-specific IgG subclass responses were measured by standard ELISA techniques using bovine CII adsorbed to ELISA plates as the immobilized phase.

The animals in the gene gun-only group exhibited no detectable CII-specific IgG activity, but low IgM titers were seen. This was surprising in view of the relatively strong responses that gene gun immunizations normally elicit (using plasmid DNA vectors), and could be related to the fact that gene gun delivery is largely an intracellular process.

In contrast, the two groups of animals challenged with CII/Freund's (delivered via gen gun or subcutaneously) exhibited significant CII-specific IgG

activity after challenge, but diff red in the relative proportions of IgG subclasses, depending on whether or not they received prior gene gun CII treatments. Arthritic animals challenged with CII/Freund's exhibited high levels of CII-specific IgG1, IgG2a, and IgG2b. However, the gene gun-protected animals that failed to develop disease in response to CII/Freund's challenge exhibited a preponderance of CII-specific IgG1, and reduced levels of IgG2a, and IgG2b responses.

These findings are consistent with the conclusion that epidermal gene gun immunizations desensitize animals toward autoantigens, as well as with the hypothesis that such desensitization may be associated with protection in the rodent CIA and AA models of human rheumatoid arthritis.

Example 2

Gene Gun-Mediated Delivery of a Gene Encoding Hsp65 from *M. tuberculosis*

A further experiment was carried out to assess the efficacy of a gene gun-mediated DNA immunization approach to prevention of autoimmune disease. Briefly, two successive Hsp65 DNA treatments to the epidermis, three weeks apart, protected 100% of mice from CIA development after CII/Freund's challenge. 77% of control animals that did not receive Hsp65 gene gun treatments developed disease within 6 weeks of challenge. The Hsp65 antigen was obtained, prepared, and delivered using techniques as described above in Example 1. In this regard, the nucleic acid sequence and corresponding amino acid sequence for the 65 kDa heat shock protein of *M. tuberculosis* (Hsp65) are known, and are disclosed herein as SEQ ID NOs 1 and 2.

Thus, this is the first demonstration where Hsp65 immunization has resulted in complete protection against CIA in mice. In an earlier report, the immunodominant Hsp65 180-188 epitope (the immunodominant epitope responsible for both disease induction and protection in AA) failed to provide protection against CIA in mice, but was protective against AA (Yang et al., *Clin. Exp. Immunol.* 87:99-104 (1992)). The ability to provide complete protection against CIA in this case suggests that active immunotherapy via epidermal gene transfer has significant potential to modulate existing anti-self inflammatory responses.

To correlate the above-described Hsp65 DNA-mediated protection with antigen-specific immune responses in treated animals, CII specific IgG subclass responses were measured in both the pretreated and control challenge groups; these data are shown in Figure 3. More particularly, serum samples from mice challenged with bovine CII in Freund's adjuvant were collected 42 days after challenge. Half of the animals received two Hsp65 gene gun deliveries to the abdominal epidermis, at 28 and 14 days prior to challenge, respectively.

CII-specific IgG subclass responses were measured by standard ELISA techniques using adsorbed bovine CII as the immobilized phase. Although the Hsp65 DNA pretreatment, in theory, would not directly affect the induction of humoral and cellular immune responses to CII during challenge, the cytokine balance in these animals could have been altered, thus affecting the animals' recognition of the CII challenge in an indirect manner. As can be seen in the data of Figur 3, a small but statistically significant reduction in CII-specific IgG1 and IgG2a antibody levels, was seen in treated animals versus

controls, which results are consistent with a shift in the cytokine balance in treated animals.

Example 3

Prevention of CIA with a Gene Encoding CII

The data described in Examples 1 and 2 suggest that peptide antigens, or nucleic acids encoding the same, delivered to the epidermis via intracellular particle delivery, provide an effective means of stimulating anti-inflammatory immune responses. Since rheumatoid arthritis is a disease involving inflammatory autoimmune recognition, active immunotherapeutic approaches based on gene gun-mediated vector delivery to the epidermis are indicated in the prevention of rheumatoid arthritis.

Protection against CIA development may also be achieved via delivery of a CII expression vector. Comparisons between gene gun delivery of protein- and DNA-based immunogens in murine influenza and hepatitis B immunization models have demonstrated that DNA immunization leads to significantly stronger humoral and cytotoxic T lymphocyte responses.

In order to optimize such particle mediated delivery parameters, the following studies are carried out. A human type II procollagen cDNA clone is inserted into a clinical grade CMV intron A-based expression construct. A systematic study examining the efficacy of various viral and cellular promoters in a variety of tissues *in vivo* following gene gun-mediated DNA delivery has demonstrated the enhanced effectiveness of the human CMV immediate early promoter in skin. Cheng et al., *Proc. Natl. Acad. Sci. USA* 90:4455-4459 (1993). Thus, the hCMV promoter, with and without its associated intron A sequence, has been the promoter of choice for most DNA immunization experiments.

To optimize the CII vector delivery regimen, a systematic study involving variations in the number and timing of immunizations is carried out. This study is important since earlier studies employing antigen expression vectors from several microbial pathogens have demonstrated dramatic effects on the quality of resulting immune responses, when both the resting period between doses and the total number of doses were varied. Repetition of multiple dosages with short resting periods therebetween likely favors the development of tolerance-type Th2 immune responses.

Gene gun DNA dosages in excess of 0.025 to 0.05 μg per shot generally yield the maximum level of expression obtainable in a given skin delivery site. Therefore, to increase the dosage beyond this point, the number of shots per immunization are increased.

To examine single shot DNA doses ranging from 1.0 μg to 0.001 μg per dose, the treatment protocol includes a group of animals that receive three shots per immunization. The number of treatments and the length of resting periods between treatments are based on results of the above study investigating these variables. The regimen chosen is the one resulting in CII-specific immune responses exhibiting the greatest anti-inflammatory character and protection. The short resting period groups will likely exhibit the most favorable responses.

To attempt to enhance Hsp65 expression, an appropriate CMV intron A vector can be used. In addition, a heterologous signal peptide coding sequence can be inserted into the CMV Hsp65 vector to enhance immunogenicity via secretion.

As discussed above for the human CII vector optimization studies, it is important to determine how the number of Hsp65 DNA doses and the timing between

doses affect Hsp65-specific immune responses prior to challenge, and how the various immunization schedules affect both CII- and Hsp65-specific immune responses following CII/Freund's challenge. Therefore, a systematic evaluation of variables including the number of immunizations (1 to 4), the length of resting periods between immunizations (1 week to 2 months), and the Hsp65 DNA dosage (1.0 to 0.001 μg) is performed as described above.

Endpoints in this study include the incidence of arthritic disease and disease score and CII- and Hsp65-specific IgG isotype profiles, cytokine profiles, and proliferative T cell responses. Immunization protocols that favor the induction of maximum IgG1, TGF-beta, IL-4, and IL-10 production levels may correlate with enhanced protective effects.

Example 4

Immunization of Animals with DNA Coding for Myelin Basic Protein

This example illustrates the effectiveness of gene gun vaccination with a plasmid encoding the gene for myelin basic protein (MBP), an encephalitogen, in preventing the induction of EAE in rats.

The rat MBP gene was cloned in the expression vector pJW4303 as follows: A 599 bp *Bam*HI-*Hinc*II fragment was isolated from pMBP-1 (positions 58-657) (Roach et al., *Cell* 34:799 (1983)). Two synthetic oligonucleotides:

5'-CTAGCATGGCATCACAGAAGAGACCCTCACAGCGACACG (SEQ ID NO.); and

5'-GATCCGTGTCGCTGTGAGGGTCTCTTCTGTGATGCCATG (SEQ ID NO.) were annealed and ligated to the fragment to reconstitute the N-terminal coding sequence and introduce a *Nhe*I site at the 5' end.

The product was cloned in frame with TPA leader peptide encoded by the vector pJW4303, prepared by the method of Lu et al., *J. Virol.* 70:3978 (1996), between the *Nhe*I and the *Bam*HI sites of the plasmid. The resulting construct was designated pJWMBP. A protein of 18.5 kD was detected by Western blot analysis, using a rabbit polyclonal antibody specific for rat MBP, in 143B cells transfected with pJWMBP. Closed circular plasmid DNA was purified from transformed DH5a-competent cells by CsCl-ethidium bromide gradient ultracentrifugation using standard protocols as described by Sambrook et al., *Molecular Cloning-A Laboratory Manual*, 2d Ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York, 1989.

DNA concentration was determined by optical density at 260nm and confirmed by agarose gel analysis comparing standards of known concentration with varying amounts of restriction endonuclease fragments of the plasmid. There was no detectible chromosomal DNA in the plasmid preparations as assessed by gel analysis. After ethanol precipitation, the DNA plasmid was aliquoted and stored at -70°C and when required, was re-precipitated and dissolved in normal saline at 4°C.

The plasmid DNA was attached to gold particles by adding 100 µl of 0.1 M spermidine (Sigma Chemical Co.) to a 1.5 ml centrifuge tube containing 50 mg of gold powder (0.95 µm diameter gold powder). The plasmid and gold were precipitated by adding 200 µl of 2.5 M $CaCl_2$ solution during vortex mixing and after settling for 10 minutes, the precipitate was washed extensively with absolute ethanol and resuspended in EtOH at 7.0 mg gold/ml. The DNA/gold preparation was then loaded into a gene gun sample cartridge using known techniques, and then delivered

to experimental animals using an electric discharge particle acceleration device as follows.

Specific pathogen-free female Lewis rats (obtained from the Animal Breeding Establishment, The Australian National University) were anesthetized with ethyl ether and their abdomens shaved with electric hair clippers. Animals were placed on their backs and their abdomens gently stretched by extending the fore and hind limbs. A hand held electric discharge Accell particle bombardment device (Auragen, Inc. Middleton, Wis.) was used for gene gun inoculations in a similar procedure to that described by Eisenbraun et al., *DNA Cell Biol.* 12:791 (1993). This procedure was repeated at 3-5 weekly intervals for a total of 5 deliveries according to the protocol shown in Figure 4.

All animals were bled 3-4 weeks after each immunization, as shown in Figure 4 and total Ig, IgG1, and IgG2a antibody levels were determined in the rat sera using the following ELISA method. Flat bottomed, 96 well ELISA plates (Titertek) were coated with 50 µl of 10 µg/ml MBP diluted in 0.01M PBS and incubated at 4°C overnight. The plates were washed 3 times in PBST (0.01M PBS, 0.1% TWEEN 20) and blocked with 100 µl/well of 3% bovine serum albumin (BSA) in PBST for 1 hr at room temperature. After washing with PBST 3 times, 50 µl of the diluted sera sample and a rat-anti MBP sera diluted in 1% BSA-PBST was transferred to each well. The plates were incubated either overnight at 4°C or 2h at room temperature, washed 6 times and 50 µl of the appropriate dilution of each Ig antibody was added to each plate and incubated for 1 hr. After washing, 50 µl of the ABTS peroxidase substrate system (Kirkegaard and Perry Laboratories, Inc.) was added to each well. After 7 minutes, the reaction was stopped by adding 50 µl of 1% soduim dodecyl sulphate (SDS). Optical density was measured using a microplate reader

at 405 nm with reference to 490 nm. Levels of antibody were determined using an endpoint calculated by measuring three times the mean value of the background optical density and the standard deviation. A positive response was considered to be detection of antibody at dilutions greater than those of corresponding control animals.

In some treated rats, MBP-specific antibodies were detected after they had received only three immunizations with pJWMBP, and by the 5th immunization, 8 of 9 rats had seroconverted (see Table 1 below). In rats that responded, circulating anti-MBP antibody titres ranged from 1/160-1/2560. As outlined in Table 1, analysis of the antibody isotype showed that animals immunized with pJWMBP produced primarily IgG1 responses. Only one animal in this group produced any IgG2a, and this was at a low level (titer: 1/80).

This isotypic response is consistent with previous findings by the present inventors that intradermal DNA immunization of autoantigens using a gene gun to deliver genes preferentially stimulates IgG1 responses, while immunization with intramuscular injections promotes IgG2a responses.

Table 1

Antibody responses of pJWMBP immunized animals challenged with MBP in CFA

Plasmid	IgG1 anti-MBP antibody titre Days after challenge				
	0	7	20	Ratio of IgG1/IgG2a	Disease Status
pJW MBP	320	960	40,960	64	NC
	<20	<20	20,480	8	C
	640	1,280	81,920	32	NC
	1,280	1,280	81,920	256	NC
	640	1,280	30,720	24	NC
	640	480	40,960	32	NC
	2,560	5,120	163,840	64	NC
	2,560	20,480	327,680	256	NC
	160	10,240	81,920	32	NC
pJW control	<20	40	20,480	8	C
	<20	<20	2,560	1.3	NC
	<20	<20	5,120	2	C
	<20	<20	15,360	12	C
	<20	<20	10,240	5.3	C
	<20	<20	10,240	4	NC
	<20	<20	40,960	5.3	C
	<20	<20	5,120	2	C
Untreated	<20	<20	10,240	4	C
	<20	<20	20,480	8	C
	<20	<20	10,240	2	C
	<20	<20	5,120	4	C

C Clinical Disease
NC No Clinical Disease

As shown in Tables 1 and 2, pJWMBP protected 8 of 9 animals from the development of autoimmune encephalomyelitis after challenge with MBP in complete Freund's adjuvant. One animal developed symptoms of

EAE. However, this animal was also the one which failed to seroconvert to MBP after gene gun immunization, suggesting that priming had not been successful. Seven out of 9 animals in the pJW control plasmid immunized group developed clinical symptoms of EAE within 14 days of challenging with MBP in complete Freund's adjuvant. Four out of four animals in the control, non-immunized group, developed EAE.

Table 2
Prevention of EAE by immunizing animals with pJW MBP DNA

			Histological score†	
Group	Sick/ Total	Mean Maximum Clinical Score	Animals Not Exhibiting Clinical Disease	Animals Exhibiting Clinical Disease
Naive Control	4/4	3.2	-	3.8, 1.0, 3.1, 4.7
pJW Control DNA	6/8	2.1	2.5, 4.5	5.2, 4.1, 5.1, 2.3, 5.5, 3.1
pJW MBP DNA	1/9	0.3	0, 0, 0, 0, 0.8, 0.13, 0.25, 0.4, 2.5	*2.5

† Lesions counted per section (60 sections examined).
* Animal that failed to seroconvert after immunization.

Animals were sacrificed on day 20 and their brains and spinal cords were collected for histological sectioning and staining. Histological sections from the pJW control and nonimmunized control groups showed extensive inflammatory lesions in the brain and spinal cord, as noted in the histological scores shown in Table 2. Two animals from the pJW control plasmid-immunized group did not develop clinical signs of disease, despite the fact that they were found to have severe CNS inflammation. Histological sections of the pJWMBP-immunized group revealed that 7 of the 8 protected animals showed

either no inflammatory response, or mild lesions in the meninges. One animal from this group, despite not developing clinical disease, exhibited extensive lesions both in the parenchyma and meninges.

The results outlined in Table 2 clearly show that the majority of animals (8 of 9) immunized with MBP DNA via gene gun-administration were protected from developing brain and spinal cord lesions and the associated clinical disease symptoms of EAE. The induction of MBP specific IgG1 antibody and the lack of an IgG2a response suggests that gene gun-immunization resulted in a shift toward a Th2 type of response and suppression of a cytotoxic immune response.

However, within 7 days of challenge with MBP in complete Freund's adjuvant, there was a significantly greater increase in the level of circulating IgG1 antibody against MBP in animals which had been immunized with pJWBMBP and protected against disease compared to animals from either of the control groups. All groups of animals produced IgG1 anti-MBP antibody by day 20, a time when all control animals had recovered from disease, however, significantly higher levels were observed in those animals which were protected from developing EAE. The ratio of IgG1/IgG2a antibody in protected animals given pJWMBP was consistently higher than in the control groups of animals exhibiting clinical EAE. This suggests that gene gun vaccination with pJWMBP desensitized the animals to MBP and decreased the cytotoxic immune response.

Example 5

Vaccination Against Myelin Oligodendrocyte Glycoprotein

This example demonstrates gene gun vaccination with a plasmid encoding the gene for the encephalitogen, myelin oligodendrocyte glycoprotein in preventing the induction of EAE in rats.

In an essentially identical manner to that described for the gene encoding myelin basic protein in Example 4, the human myelin oligodendrocyte glycoprotein (MOG) gene (Hilton et al., *J. Neurochem.* 65:309-318 (1995)) is cloned into the expression vector pJW4303. The plasmid DNA is attached to gold particles as described in Example 4. Specific pathogen-free female Lewis rats, as described in Example 4, are given gene gun inoculations with the plasmid containing cDNA encoding the human MOG antigen. This procedure is repeated 3 to 5 times at weekly intervals in a similar protocol to that depicted in Figure 4. Rats vaccinated in this manner are not expected to develop autoimmune encephalomyelitis.

Example 6

Vaccination Against Proteolipid Protein

This example demonstrates gene gun vaccination with a plasmid encoding the gene for the encephalitogen, proteolipid protein (PLP) in preventing the induction of EAE in rats.

Using the molecular biology techniques detailed in Example 4 with respect to preparation of the myelin basic protein nucleic acid molecule, the human PLP gene (Nadon et al., *Development* 110:529-537 (1990)) is cloned into the expression vector pJW4303. The plasmid DNA is attached to gold particles as described in Example 4. Specific pathogen-free female

Lewis rats, as described in Example 4 are given gene gun inoculations with the plasmid containing DNA encoding the human MOG antigen. This procedure is repeated 3 to 5 times at weekly intervals for a total of 3-5 deliveries in a similar protocol to that shown in Figure 4. Rats vaccinated in this manner will not develop autoimmune encephalomyelitis.

Example 7

Vaccination with the Entire cDNA Library of Human Oligodendrocytes

This example demonstrates gene gun vaccination with a plasmid encoding the entire cDNA library of human oligodendrocytes in preventing the induction of EAE in rats.

Human oligodendrocytes are isolated and grown in culture according to the method of Zhou et al., *J. Neurosci. Res.* 42:504-515 (1995) and an expression cDNA library is prepared from these cells. Total RNA is prepared from oligodendrocytes by extraction with RNAzol, according to the manufacturer's (Tel-Test, Inc.) instructions. Messenger RNA is isolated using the "mRNA purification kit" (Pharmacia, 27-9258-01).

First strand cDNA is synthesized from 2 μg mRNA, with 100 ng of random hexamer primer, using the "SuperScript Preamplification System for First Strand cDNA Synthesis" (GIBCO BRL, 18089-011). Second strand cDNA is synthesized by a modification of the method of Gubler and Hoffman, *Gene* 25:263-269 (1983). All reagents used in this preparation are from GIBCO BR. The first strand cDNA reaction mixture (20 ul) is added
to a premix of 16 μl of 10X *E.coli* ligase buffer, 2.4 μl 10 mN cNTP mix and H_2O, on ice. RNAse H (1.6 U), DNA polymerase I (40 U) and *E. coli* DNA ligase (40 U)

are added and the reaction (final volume, 160 μl) is incubated for 1 hr at 16°C, then 1 hr at room temperature. Glycogen carrier (10 mg; Boehringer-Mannheim) is added and the cDNA purified by phenol extraction and 2 ether extractions, followed by ethanol precipitation with 2 M ammonium acetate, washed in 70% ethanol and resuspended in 20 ml 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (TE). Double stranded cDNA (1 μl) is digested with 12 U of Sau3A (New England Biolabs) at 37°C overnight. The restriction enzyme is inactivated at 70°C for 30 min, and the DNA is ethanol precipitated, washed with 70% ethanol and resuspended in 20 μl TE buffer.

CMV-GH-F1, -F2, or -F3 vectors (20 μg) are digested with BamHI or BglII according to the method of Durry et al., *Nature* 377:632-635 (1995) and treated with 2U of calf alkaline phosphatase (30 min at 37°C, 60 min at 50°C, 10 min at 75°C; Boehringer Mannheim). The vectors were extracted with phenol/chloroform, ethanol precipitated and electrophoresed on 1% agarose (low melting temperature, DNA grade agarose, Progen industries) with 0.1 μg/ml ethidium bromide, excised in the minimum possible volume of agarose and melted at 65°C before adding to the ligation mix. The *Sau*3A digested cDNA (2 μl) is ligated to 100 ng of vector (in agarose) with 5U of T4 DNA ligase (Boehringer Mannheim) in 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP in a final volume of 20 μl, overnight at 16°C.

Electrocompetent *E. coli* are transformed with the ligated cDNA, using a Biorad Gene Pulser (25 uF, 2.5 kV) with Pulse Controller at 200 Ohms and 0.2 cm cuvettes (according to a protocol from Biorad). The transformation efficiency is approximately 5 X 10^8 colonies per μg DNA. 2 μl *Sau*3A digested cDNA

(equivalent to 10 ng mRNA) gives from 10 to 25,000 colonies.

The cDNA library is amplified. Thus, after electrotransformation, the library is plated out at high density on L-broth containing 100 μg/ml ampicillin (approximately 30,000 per 9.5 cm square plate). The colonies are grown overnight to a diameter of 0.2-0.3 mm, and rinsed from the plate in a total of 10 ml L-broth per plate. L-broth from several plates is combined, glycerol added to 15% v/v, and the library stored in 1 ml aliquots at -80°C. Separate libraries are generated for each of the 3 CMV-GH vectors.

DNA is prepared in the following manner. L-broth, containing 100 μg/ml ampicillin (70 ml) is inoculated with a 1 ml library aliquot and grown overnight at 37°C with vigorous shaking. The next day, 1 liter baffled flasks containing 500 ml L-broth, containing 100 μg/ml ampicillin are inoculated with 10 ml of overnight culture and grown overnight at 37°C. Closed circular plasmid DNA is purified by CsCl-ethidium bromide gradient ultracentrifugation and attached to gold beads as described in Example 4. Immunization of rats with the Acell particle bombardment device (Auragen, Inc., Middleton, Wis) is used according to the protocol in Example 4.

Accordingly, novel methods for treating or preventing autoimmune disease have been described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

SEQUENCE LISTING

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4380 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 252..1874
(D) OTHER INFORMATION: /product= "65 kDa heat shock protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCGAACGAGG GGCGTGACCC GGTGCGGGGC TTCTTGCACT CGGCATAGGC GAGTGCTAAG 60

AATAACGTTG GCACTCGCGA CCGGTGAGTG CTAGGTCGGG ACGGTGAGGC CAGGCCCGTC 120

GTCGCAGCGA GTGGCAGCGA GGACAACTTG AGCCGTCCGT CGCGGGCACT GCGCCCGGCC 180

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AGCGTAAGTA GCGGGGTTGC CGTCACCCGG TGACCCCCGT TTCATCCCCG ATCCGGAGGA 240

ATCACTTCGC A ATG GCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC  290
              Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg
                1               5                  10

GGC CTC GAG CGG GGC TTG AAC GCC CTC GCC GAT GCG GTA AAG GTG ACA   338
Gly Leu Glu Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr
     15                  20                  25

TTG GGC CCC AAG GGC CGC AAC GTC GTC CTG GAA AAG AAG TGG GGT GCC   386
Leu Gly Pro Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala
 30                  35                  40                  45

CCC ACG ATC ACC AAC GAT GGT GTG TCC ATC GCC AAG GAG ATC GAG CTG   434
Pro Thr Ile Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu
                 50                  55                  60

GAG GAT CCG TAC GAG AAG ATC GGC GCC GAG CTG GTC AAA GAG GTA GCC   482
Glu Asp Pro Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala
           65                  70                  75

AAG AAG ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG GCC ACC GTG   530
Lys Lys Thr Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val
      80                  85                  90

CTG GCC CAG GCG TTG GTT CGC GAG GGC CTG CGC AAC GTC GCG GCC GGC   578
Leu Ala Gln Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly
 95                  100                 105

GCC AAC CCG CTC GGT CTC AAA CGC GGC ATC GAA AAG GCC GTG GAG AAG   626
Ala Asn Pro Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Lys
110                 115                 120                 125

GTC ACC GAG ACC CTG CTC AAG GGC GCC AAG GAG GTC GAG ACC AAG GAG   674
Val Thr Glu Thr Leu Leu Lys Gly Ala Lys Glu Val Glu Thr Lys Glu
                130                 135                 140

CAG ATT GCG GCC ACC GCA GCG ATT TCG GCG GGT GAC CAG TCC ATC GGT   722
Gln Ile Ala Ala Thr Ala Ala Ile Ser Ala Gly Asp Gln Ser Ile Gly
           145                 150                 155

GAC CTG ATC GCC GAG GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC ATC   770
Asp Leu Ile Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile
      160                 165                 170

ACC GTC GAG GAG TCC AAC ACC TTT GGG CTG CAG CTC GAG CTC ACC GAG   818
Thr Val Glu Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu
 175                 180                 185

GGT ATG CGG TTC GAC AAG GGC TAC ATC TCG GGG TAC TTC GTG ACC GAC   866
Gly Met Arg Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp
190                 195                 200                 205

CCG GAG CGT CAG GAG GCG GTC CTG GAG GAC CCC TAC ATC CTG CTG GTC   914
Pro Glu Arg Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val
                210                 215                 220

AGC TCC AAG GTG TCC ACT GTC AAG GAT CTG CTG CCG CTG CTC GAG AAG   962
Ser Ser Lys Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys
           225                 230                 235

GTC ATC GGA GCC GGT AAG CCG CTG CTG ATC ATC GCC GAG GAC GTC GAG  1010
Val Ile Gly Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu
      240                 245                 250
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GGC GAG GCG CTG TCC ACC CTG GTC GTC AAC AAG ATC CGC GGC ACC TTC  1058
Gly Glu Ala Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe
    255                 260                 265

AAG TCG GTG GCG GTC AAG GCT CCC GGC TTC GGC GAC CGC CGC AAG GCG  1106
Lys Ser Val Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala
270                 275                 280                 285

ATG CTG CAG GAT ATG GCC ATT CTC ACC GGT GGT CAG GTG ATC AGC GAA  1154
Met Leu Gln Asp Met Ala Ile Leu Thr Gly Gly Gln Val Ile Ser Glu
                290                 295                 300

GAG GTC GGC CTG ACG CTG GAG AAC GCC GAC CTG TCG CTG CTA GGC AAG  1202
Glu Val Gly Leu Thr Leu Glu Asn Ala Asp Leu Ser Leu Leu Gly Lys
            305                 310                 315

GCC CGC AAG GTC GTG GTC ACC AAG GAC GAG ACC ACC ATC GTC GAG GGC  1250
Ala Arg Lys Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly
        320                 325                 330

GCC GGT GAC ACC GAC GCC ATC GCC GGA CGA GTG GCC CAG ATC CGC CAG  1298
Ala Gly Asp Thr Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Gln
    335                 340                 345

GAG ATC GAG AAC AGC GAC TCC GAC TAC GAC CGT GAG AAG CTG CAG GAG  1346
Glu Ile Glu Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu
350                 355                 360                 365

CGG CTG GCC AAG CTG GCC GGT GGT GTC GCG GTG ATC AAG GCC GGT GCC  1394
Arg Leu Ala Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala
                370                 375                 380

GCC ACC GAG GTC GAA CTC AAG GAG CGC AAG CAC CGC ATC GAG GAT GCG  1442
Ala Thr Glu Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala
            385                 390                 395

GTT CGC AAT GCC AAG GCC GCC GTC GAG GAG GGC ATC GTC GCC GGT GGG  1490
Val Arg Asn Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly
        400                 405                 410

GGT GTG ACG CTG TTG CAA GCG GCC CCG ACC CTG GAC GAG CTG AAG CTC  1538
Gly Val Thr Leu Leu Gln Ala Ala Pro Thr Leu Asp Glu Leu Lys Leu
    415                 420                 425

GAA GGC GAC GAG GCG ACC GGC GCC AAC ATC GTG AAG GTG GCG CTG GAG  1586
Glu Gly Asp Glu Ala Thr Gly Ala Asn Ile Val Lys Val Ala Leu Glu
430                 435                 440                 445

GCC CCG CTG AAG CAG ATC GCC TTC AAC TCC GGG CTG GAG CCG GGC GTG  1634
Ala Pro Leu Lys Gln Ile Ala Phe Asn Ser Gly Leu Glu Pro Gly Val
                450                 455                 460

GTG GCC GAG AAG GTG CGC AAC CTG CCG GCT GGC CAC GGA CTG AAC GCT  1682
Val Ala Glu Lys Val Arg Asn Leu Pro Ala Gly His Gly Leu Asn Ala
            465                 470                 475

CAG ACC GGT GTC TAC GAG GAT CTG CTC GCT GCC GGC GTT GCT GAC CCG  1730
Gln Thr Gly Val Tyr Glu Asp Leu Leu Ala Ala Gly Val Ala Asp Pro
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GTC AAG GTG ACC CGT TCG GCG CTG CAG AAT GCG GCG TCC ATC GCG GGG  1778
Val Lys Val Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Gly
    495                 500                 505
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CTG TTC CTG ACC ACC GAG GCC GTC GTT GCC GAC AAG CCG GAA AAG GAG  1826
Leu Phe Leu Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Glu
510                 515                 520                 525

AAG GCT TCC GTT CCC GGT GGC GGC GAC ATG GGT GGC ATG GAT TTC TGA  1874
Lys Ala Ser Val Pro Gly Gly Gly Asp Met Gly Gly Met Asp Phe  *
                530                 535                 540

CCCCGGCGAG AAGTCGCAGC GAGGAGCCCG GTCCCTTTGT GGGGCCGGGC TCCTCTGGTT 1934

GGGAGCTACG GTACCGAGAA CACCACGCAG TCGTGTAGGC AACCTTTGGC CGCTGTGGGC 1994

GAGTCGGGGG CCGCGTCTCG GTGCAGCAGC GCGCGGATGG GTACGACACC GCAGCGGGCG 2054

GTGTCGTCAT CGGGGCCTGC GTCCGACGCC TGGGCACGGC CGTCGACGAT CAGCGAGTAG 2114

CCGCTAGGAT CGGATGGCGG CCACAACAGG GTGACTTCGC TGCGGTGGGC CAGGTTTTGC 2174

CGCGTACGAC CCCCGATCAG GCCGACGTCG ACCACTGCCC GGGGTCCATC GGGGCCGTCG 2234

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GCCCGATCCG CGGGCTGGCG TAGGCGGATT CGCGGTCGGG GCTCGGGTAG AAGTTCGACT 2474

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TTGCGCCCGA GCCAGCCGCG GCACTGCCGC TACCGGGGTT CGGGTTGCCT GAGTCCAGGC 2654

CGCCAACAGG AGCACTGGCC GGGGCGGCGA CGGGCGTGTT GGTCAGGCCC GAGTTGAGGA 2714

CGTTCGCCAG GCCGTGTTGG AGACCGCCCG TTGATCCGAG GGCGGAGGCG AGGATGCCCG 2774

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CCTCCGAGCC AGCCGCGCTT CCTAAGGCGG CGTTTTGCAT CCCCGCGTTC CAGAAGCTGG 2894

TGTTGAGGCT GCCTGCGCTG CCGAGGCCCG CGTTGATTGT CCCCGAGGTC CCGATGCCGC 2954

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AACCCATCTG GTGATCACCG GTGATCCCGA ACCCGATATT CCCGCTACCG GTGTTGCCGA 3134

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TGCCGATGTT GCCGGTGCCG GTGTTGCCGC TGCCGATGTT GTTGTTGCCG ATGTTGTTGT 3254

TGCCGATGTT GTTGTTGCCG ATGTTGCCGC TGCCGGTGTT GCCGAAGCCC AGATTGATCT 3314

GGCCGTTCTT GCCGATGTCG ATGCCGAGGT TCCGCAAGAC CTGCTGCCAG GGCGCCAGTT 3374

GTGCGACGGC CGCAGACGCA TCGAAGTGGT AACCAGCCAT CGCCGCCACG TCCAATGCCC 3434

ACATTTGCTC GTATGCCGCC TCGACGTCCA TGAGCGCCGG AGCGTTCTGC CCAAACCAGT 3494

TCGTAGCTGC CAGCAGCTGC ATCAGGCCAC GATTGGCCGC TACCACTGCC GGCTGCACGG 3554

TGGCCGCCAG CGCCGCCTCG AACGCGGTCG CTGTTGCCAT GGCCTGTGCG GCCGCTTGTT 3614
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CCGCCTGCGC TGCCGCCGTG CTGAGCCAGG CTAGGTACTG GGTTGCGACG GCCATCATCG 3674

CCGCCGCGGA CGGACCCAGC CAGGCGCCAC TAGTCAGTTC GGATGTGACG GAGCCAAGCG 3734

ACGCTATTGA CGCGAGCAAT TCTTCGGCCA GCTCGCCCCA GGCGGTGGCC GCAGCAATTA 3794

GCGGTCCCGA CCCGGGACCG GCAAACATCA GTGCCGAATT GATCTCTGGC GGCAACCACG 3854

CAAAATGCGG GCTTGTCAGC CGATCCAACT TAACTGTCAG CGACCGTTGC CGTGGCGGTA 3914

TCGGCACTTC AATACCACTC ATCTTTGGGG TCATCTTTGG AGCGCCCCTA GGAACCGCCA 3974

GCTTACCTAG TCCCGGGTAG GGGCCGACTG GCGGCCGGGA TGCAGCTGAG GGTCTGCCAC 4034

CTGCCCCGTA ATGTCGCTGG TATGGCAAGC ACCGACGCCG CGGCCCAAGA GTTGCTCCGC 4094

GACGCGTTCA CCCGGTTGAT CGAACATGTC GACGAACTCA CCGACGGCCT CACCGACCAA 4154

CTCGCCTGCT ACCGCCCGAC CCCCAGCGCC AACAGCATTG CGTGGCTGCT CTGGCACAGC 4214

GCCCGGGTGC AGGATATACA GGTCGCCCAT GTGGCCGGCG TGGAAGAGGT GTGGACCCGC 4274

GACGGTTGGG TGGACCGCTT TGGGTTAGAT CTGCCGCGGC ACGACACCGG ATATGGACAC 4334

CGTCCCGAGG ATGTGGCGAA GGTACGGGCA CCCGCCGACG GAATTC 4380

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 541 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
1 5 10 15

Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
20 25 30

Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
35 40 45

Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
50 55 60

Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
65 70 75 80

Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
85 90 95

Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
100 105 110

Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Lys Val Thr Glu
115 120 125

Thr Leu Leu Lys Gly Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ala
130 135 140

Ala Thr Ala Ala Ile Ser Ala Gly Asp Gln Ser Ile Gly Asp Leu Ile
145 150 155 160

Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
165 170 175

Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
180 185 190

Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Pro Glu Arg
195 200 205

Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys
210 215 220

Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gly
225 230 235 240

Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu Ala
245 250 255

Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys Ser Val
260 265 270

Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala Met Leu Gln
275 280 285

Asp Met Ala Ile Leu Thr Gly Gly Gln Val Ile Ser Glu Glu Val Gly
290 295 300

Leu Thr Leu Glu Asn Ala Asp Leu Ser Leu Leu Gly Lys Ala Arg Lys
305 310 315 320

Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly Ala Gly Asp
325 330 335

Thr Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Gln Glu Ile Glu
340 345 350

Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala
355 360 365

Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu
370 375 380

Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn
385 390 395 400

Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly Gly Val Thr
405 410 415

Leu Leu Gln Ala Ala Pro Thr Leu Asp Glu Leu Lys Leu Glu Gly Asp
420 425 430

Glu Ala Thr Gly Ala Asn Ile Val Lys Val Ala Leu Glu Ala Pro Leu
435 440 445

Lys Gln Ile Ala Phe Asn Ser Gly Leu Glu Pro Gly Val Val Ala Glu
450 455 460

Lys Val Arg Asn Leu Pro Ala Gly His Gly Leu Asn Ala Gln Thr Gly
465 470 475 480

Val Tyr Glu Asp Leu Leu Ala Ala Gly Val Ala Asp Pro Val Lys Val
485 490 495

Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Gly Leu Phe Leu
500 505 510

Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Glu Lys Ala Ser
515 520 525

Val Pro Gly Gly Gly Asp Met Gly Gly Met Asp Phe *
530 535 540

We claim:

1. A method for treating or preventing an autoimmune disease in a mammal comprising the steps of:

(a) providing a particle coated with an antigen against which an immune response is mounted in the autoimmune disease, wherein the particle is suitable for delivery into a recipient cell, the particle having an average diameter of about 0.5 to about 5 μm and a density sufficient to allow delivery into the recipient cell;

(b) delivering the particle into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

2. The method of claim 1, wherein the cytotoxic immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-2, interferon-gamma and tumor necrosis factor.

3. The method of claim 1, wherein the desensitizing immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-4, interleukin-5, interleukin-6, and interleukin-10.

4. The method of claim 1, wherein the autoimmune disease is rheumatoid arthritis.

5. The method of claim 4, wherein the antigen is selected from the group consisting of

collagen, the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, and epitopes thereof.

6. The method of claim 4, wherein the antigen is collagen, or an epitope thereof.

7. The method of claim 4, wherein the antigen is the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, or an epitope thereof.

8. The method of claim 1, wherein the autoimmune disease is multiple sclerosis.

9. The method of claim 8, wherein the antigen is selected from the group consisting of myelin basic protein, myelin oligodendrocyte glycoprotein, proteolipid protein, and epitopes thereof.

10. The method of claim 1, wherein the particle is comprised of a metal selected from the group consisting of gold and tungsten.

11. The method of claim 1, wherein the cell is a skin cell.

12. The method of claim 1, wherein the cell is a mucosal cell.

13. A method for treating or preventing an autoimmune disease in a mammal comprising the steps of:

(a) providing a nucleic acid construct which comprises a coding sequence for an antigen against which an immune response is mounted in the autoimmune disease, operably linked to control elements such that

the coding sequence can be transcribed and translated in a recipient cell;

(b) delivering the nucleic acid construct into the recipient cell of the mammal; and

(c) repeating step (b) a sufficient number of times until either a reduction in a cytotoxic immune response or a desensitizing immune response is induced in the mammal.

14. The method of claim 13, wherein the nucleic acid construct is coated onto a particle having an average diameter of about 0.5 to about 5 μm and a density sufficient to allow delivery into the recipient cell, and step (b) entails delivering the coated particle into the recipient cell.

15. The method of claim 13, wherein the cytotoxic immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-2, interferon-gamma and tumor necrosis factor.

16. The method of claim 13, wherein the desensitizing immune response is characterized by the secretion from a T-lymphocyte of one or more mediators selected from the group consisting of interleukin-4, interleukin-5, interleukin-6, and interleukin-10.

17. The method of claim 13, wherein the autoimmune disease is rheumatoid arthritis.

18. The method of claim 17, wherein the coding sequence encodes an antigen selected from the group consisting of collagen, the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65, and epitopes thereof.

19. The method of claim 17, wherein the coding sequence encodes collagen or an epitope thereof.

20. The method of claim 17, wherein the coding sequence encodes the *Mycobacterium tuberculosis* heat shock protein Mt Hsp65 or an epitope thereof.

21. The method of claim 13, wherein the autoimmune disease is multiple sclerosis.

22. The method of claim 21, wherein the coding sequence encodes an antigen selected from the group consisting of myelin basic protein, myelin oligodendrocyte protein, proteolipid protein, and epitopes thereof.

23. The method of claim 21, wherein the coding sequence encodes the entire cDNA library of human oligodendrocytes.

24. The method of claim 21, wherein the nucleic acid construct comprises an oligodendrocyte gene library comprising one or more coding sequences operably linked to control elements such that the coding sequences can be transcribed and translated in a recipient cell.

25. The method of claim 13, wherein the cell is a skin cell.

26. The method of claim 13, wherein the cell is a mucosal cell.

27. Use, in the manufacture of a medicament for treatment or prevention of an autoimmune disease

in an animal, of a particle coated with an antigen against which an immune response is mounted in the autoimmune disease, wherein the particle is suitable for delivery into a recipient cell, the particle having an average diameter of about 0.5 to about 5 μm and a density sufficient to allow delivery into the recipient cell.

28. Use, in the manufacture of a medicament for treatment or prevention of an autoimmune disease in an animal, of a nucleic acid construct which comprises a coding sequence for an antigen against which an immune response is mounted in the autoimmune disease, operably linked to control elements such that the coding sequence can be transcribed and translated in a recipient cell.

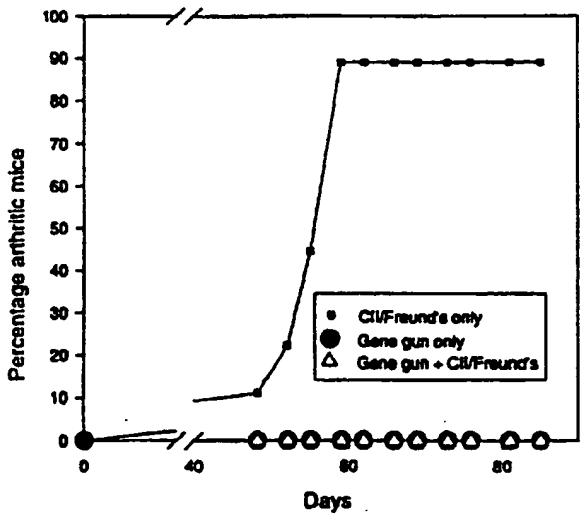
Percentage arthritic mice
100
90
80
70
60
50
40
30
20
10
0
0
40
80
80
Days
CII/Freund's only
Gene gun only
Gene gun + CII/Freund's

FIG. 1

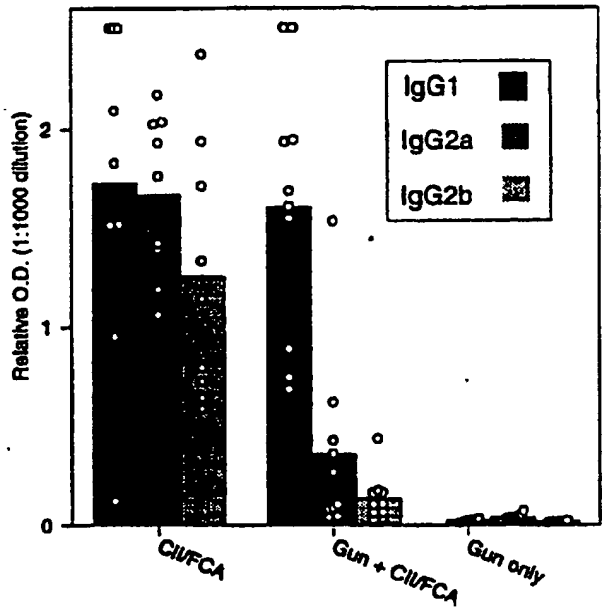
IgG1
IgG2a
IgG2b
Relative O.D. (1:1000 dilution)
0
1
2
CII/FCA
Gun + CII/FCA
Gun only

FIG. 2

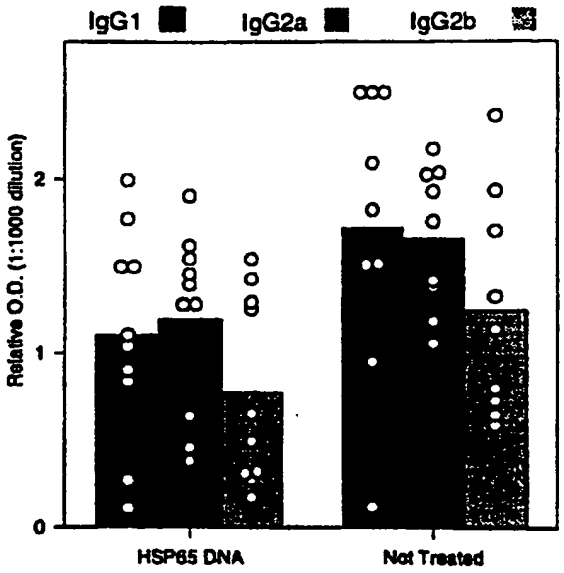
IgG1
IgG2a
IgG2b
Relative O.D. (1:1000 dilution)
0
1
2
HSP65 DNA
Not Treated

FIG. 3

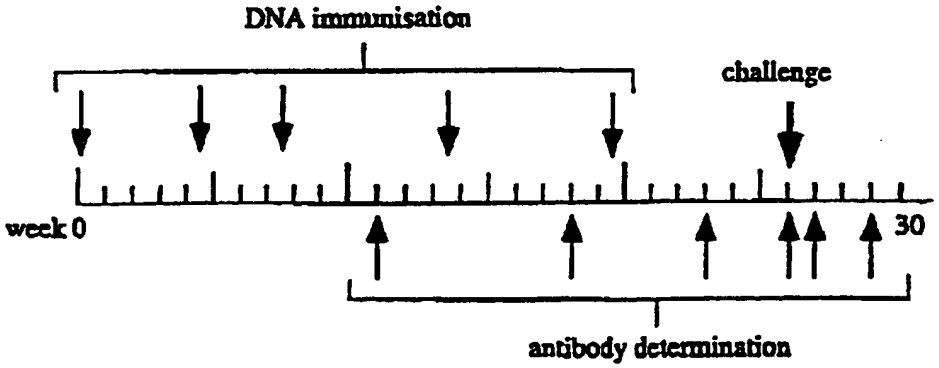
DNA immunisation
challenge
week 0
30
antibody determination

FIG. 4